REMARKS

The typographical error in claim 8 has ben corrected to meet the objection thereto.

Claim 3 has been amended to remove reference to susceptibility to heart failure and so the 35 USC 112 paragraph 2 rejection has been met.

Support for the further amendments to claim 3 to define the heart failure as "chronic symptomatic "heart failure with impairs systolic function is found in the paragraph bridging pages 7 and 8 of the specification and in Example 6 where it is pointed out that the ESPVR is widely accepted as a good indiaction of left ventricular function and the experimental results show a dose dependent improvement in left ventricular function with administration of the β_3 agonist. It is believed that these amendments together that noted above and with deletion of the reference to myocardial hypertrophy meet the written description rejection under 35 USC 112 first paragraph.

Turning now to the enablement rejection under 35 USC 112 paragraph 1, the applicants respectfully disagree with the examiner's view that one skilled in the art would need to carry out undue experimentation in order to treat those suffering from or susceptible to heart failure after reading the applicant's specification. Nevertheless in order to expedite prosecution, Claim 3 has been amended to specify more precisely the conditions which are to be treated.

The examiner has cited the Wands factors. As applied to the amended claims, the applicants comment as follows:

In respect of **Point (A)**, the claims are now amended to recite that the treatment is directed at "a human suffering from chronic symptomatic heart failure with impaired systolic left ventricular function". As noted above, the skilled person would understand the target patient group defined in this manner and, upon reading the instant specification would understand the manner in which the invention can be put into practice for such a group.

The Office Action explains concerns relating to the nature of the invention **(Point B)** and the

state of the prior art and level of predictability in the art (**Point C**). In doing so, the Examiner summarizes points made by Gauthier *et al,* (2000) and Moniotte *et al,* (2003) on pages 7-10 of the Office Action and concludes that "the art must be regarded as highly unpredictable" (page 10).

In reply, the applicants note that it is important to recognize that the relevant points made by Gauthier et al. and Moniotte *et al.* are entirely speculative in nature and that they make no claim of supportive evidence. As the Office Action highlights, those papers speculate that β_3 adrenoceptor stimulation could be beneficial in early stages but harmful in later stages of heart failure with no evidence for either speculation. It is also important that their speculations refer to effects of endogenous adrenergic agonists, not agonists used as a treatment. Anyone skilled in the art of treating heart failure would not base treatment decisions on such speculations. However, if one for the moment accepts the speculations, the present invention brings predictability/reduces risk of using adrenoceptor stimulation as a treatment.

If the speculations that β_3 adrenoceptor stimulation could be beneficial in "early stages" of heart failure reflected a practical reality, it is unlikely that there would be harm with therapeutic use of exogenous synthetic β_3 adrenoceptor agonists at such stages; it might be useful. As far as the speculation of a harmful effect of β_3 adrenoceptor stimulation in later stages of heart failure is concerned, the present invention indicates that such stimulation is expected to reverse a key pathophysiological feature of heart failure, raised levels of intracellular Na^+ in cardiac myocytes, and hence be useful at such stages.

The concept that raised myocytes Na^+ levels contribute adversely to the phenotype of heart failure was widely accepted at the time the patent application was lodged, exemplified by two comprehensive review articles addressing the evidence in humans (Pieske and Houser, 2003) and in animal models of heart failure (Pogwizd *et al.* 2003). The direct therapeutic implications of this are indicated by previous studies indicating that two evidence-based cornerstones of treatment, angiotensin converting enzyme inhibitors and aldosterone antagonists (ESC guidelines page 949 and 952) up-regulate Na^+-K^+ pump activity as studied in rabbit cardiac myocytes *ex vivo* (Hool *et al*, 1995, 1996; Mihailidou *et al.* 2000).

Additional evidence published after the patent application was lodged further supports the long-term robustness of using Na^+-K^+ pump activity measurements in rabbit cardiac myocytes to predict efficacy of treatment in human heart failure. Treatment with "β blockers" (page 951 of ESC guidelines) increases pump activity as reported in the attached Poster presented at the ESC Scientific meeting in Stockholm, 2010 (Karimi *et al*). Conversely, a clinical trial has indicated that β_1 adrenoceptor stimulation is harmful in human heart failure (The Zameterol in Severe Heart Failure Group, 1990). β_1 adrenoceptor stimulation causes Na^+-K^+ pump inhibition in rabbit cardiac myocytes (White *et al*, 2010).

As indicated above, intracellular Na^+ levels are abnormally high in heart failure. While an increase in Na^+ levels in the normal heart is expected to decrease contractility (Pieske and Houser, 2003), any increase in the failing heart is expected to decrease contractility, i.e. have a negative inotropic effect (and have other adverse effects such as promoting cardiac arrhythmias, Pieske and Houser, 2003; Pogwizd 2003). Conversely, a decrease in Na^+ levels in the failing heart might enhance contractility (and reduce arrhythmias).

The presentapplication indicates that a β_3 adrenoceptor agonist administered therapeutically can be expected to have beneficial effects in severe heart failure, contrary to the speculations of Gauthier *et al* (2000) and Moniotte *et al* (2003). It eliminates the concern about not identifying a transition from "early stages" to "later stages" in clinical practice. The safety of using β_3 adrenoceptor agonist therapeutically is supported by the *in vivo* sheep data presented in the specification.

The applicants agree with the Examiner that therapeutic use of β_3 adrenoceptor agonist would have been highly unpredictable (page 10 of Office Action) on the basis of the speculations of the prior art. "Early stages" of heart failure cannot be routinely detected nor can and a "transition to later stages" be reliably identified. The invention adds predictability because, in contrast to the speculations of the prior art, it supports a beneficial effect at "later stages". This means that the risk implied by the prior art is eliminated by the invention. While no single feature permits a straightforward identification of "later stages" of heart failure, universally accepted criteria allow identification of a target group for treatment.

In **Point (D)** the Office Action raises concerns regarding the amount of direction provided and/or the existence of working examples, in essence arising from the Examiner's concerns about perceived species variability. In reply the Applicants have the following comments. The papers by Gauthier *et al.* (2000) review inconsistent results of experimentation between different species. It is pointed out how variability in part is due to experimental methods/design. In *in vivo* studies, positive chronotopic effects (increase in heart rate) is attributed to non-selective activation of β_1 adrenergic receptors that may occur as a reflex response to a fall in blood pressure, at least in some species. The absence of a response of any measured variable is taken to indicate the absence of expression of functional β_3 adrenergic receptor expression in one species (left column, page 684, *Can J Physiol* 2000).

The applicants took into account the species issues at the time of experimentation. As indicated in the specification, especially Examples 1-4 and their respective Figures, a response of the chosen variable to the synthetic β_3 adrenergic agonist BRL 37344 and the naturally occurring agonist norepinephrine was easily demonstrated. Specificity of an agonist response of a variable is another important issue. As illustrated in Fig 4 on page 684 of Gauthier *et al.* (*Can J Physiol* 2000), it is important to ascertain that an effect attributed to the β_3 adrenergic receptor is not mediated by β_1 or β_2 adrenergic receptors because there is imperfect selectivity of the known agonist between the 3 receptors.

Example 1 in the application shows that BRL37344 in relatively low concentrations (1 and 10 nM) stimulates the Na^+-K^+ pump but that stimulation is lost at the higher concentration of 100 nM. Stimulation at the higher concentration is restored when β_1 or β_2 adrenergic receptors are blocked with nadolol (Example 2). Note, nadolol was used as an experimental tool to rule out Na^+-K^+ pump stimulation mediated by β_1 or β_2 adrenergic receptors. Similarly, nadolol, as used in Example 4, ruled out that norepinephrine-induced pump stimulation was mediated by β_1 or β_2 adrenergic receptors.

Fig 4 on page 684 of Gauthier *et al. (Can J Physiol* 2000) highlights how β_1 or β_2 adrenergic receptors in myocytes are coupled to activation of adenyl cyclase ("**AC**") that generates **cAMP** that, in turn, is known to activate cAMP-dependent protein kinase (commonly known as "PKA"). The whole-cell patch clamp technique allowed the perfusion of the intracellular

compartment with H-89 (included in patch pipette solutions), a well known inhibitor of PKA. H-89 had no effect on norepinephrine-induced Na^+-K^+ pump stimulation (Example 4) supporting the conclusion that stimulation was mediated by the β_3 adrenergic receptor rather than β_1 or β_2 adrenergic receptors. In further support of this conclusion, inclusion of a cAMP analog in pipette solution, mimicking β_1 or β_2 adrenergic receptor activation decreased pump function, and effect opposite that of β_3 adrenergic receptor agonists. The effect of the cAMP analog was abolished by H-89, indicating efficacy of the PKA inhibitor in the concentration used (Example 5).

To summarize, the studies on rabbit myocytes clearly indicates a functional response to β_3 adrenergic receptor agonists, and the concern frequently raised about the specificity of β_3 - vs. β_1 or β_2 adrenergic receptor-mediated responses (Gauthier et al (Can J Physiol 2000) was comprehensively addressed. The evidence presented in the application would be regarded by the skilled addressee as more than adequate to consider rabbit heart a "responder" and a skilled person would not have to extrapolate.

Rabbit heart had never been declared a non-responder; it just hadn't been reported at the time the patent application was submitted. As indicated above, the Examples in the application provide evidence that would classify rabbits as responders (as are humans). In support of this conclusion, Gauthier's group has subsequently published evidence indicating that rabbit cardiac myocytes are particularly good models for human conditions (Audigane *et al,* 2009). With reference to a 1998 publication by Hasenfuss *et al.* they point out that rabbit cardiac function is similar to that of human heart (Audigane *et al.*, 2009, page 401, upper left column) and summarize how the responses they describe in the article are consistent with those they had previously described in human heart (page 401, upper left column). They even imply that studies on rabbit myocytes may provide more relevant physiological information than studies on human tissue because human tissues that can be obtained is modified by advanced age and or disease (page 401, second paragraph). In the final paragraph of the Discussion (page 410) the authors conclude that the rabbit model "will be useful to facilitate understanding and further investigation of β_3-AR physiological and pathophysiological implications in cardiovascular regulation".

Much of the classification of animals into "responders" or "non-responders" is based on the effect of a β_3 adrenergic receptor agonist on cardiac contractility. As discussed by Gauthier *et al.* (*Can J Physiol* 2000) effects on the vascular bed or autonomic reflexes with secondary increases in heart rate confounds interpretation of *in vivo* studies in some animals. *Ex vivo* studies on isolated cardiac tissues are also conflicting. This is not surprising since it is widely appreciated that such *in vitro* studies are subject to a number of systematic errors and should only be extrapolated to cardiac contractility under *in vivo* conditions with care.

The data shown in Example 6 of the instant specification was obtained using a well documented large-animal (sheep) model of severe heart failure. Left ventricular end diastolic pressure-volume relationships (LVEDP) were used as an index of contractility. Measurement of LVEDP requires a large amount of invasive instrumentation, and it would be very difficult to justify such measurements in humans from an ethical perspective. It is also expensive and has not been used routinely in studies that classify animals as "responders" or "non-responders". However, LVEDP is widely regarded as the best index of contractility because it is measured *in vivo* (and hence physiologically relevant) and it is relatively independent of confounders such as heart rate and after-load (the latter reflecting peripheral vascular resistance). It is particularly suited for studying effects of inotropic agents (positive or negative, as reviewed by Burkhoff *et al*; 2005; lower left column, page H505). The effect of BRL37344 indicates that sheep are "responders", using an index more rigorous than those that have been used to classify other animals. The differential effect before and after induction of heart failure is what one would have expected from the β_3 adrenergic receptor-mediated Na^+-K^+ pump stimulation in the rabbit cardiac myocytes. This supports the use of β_3 adrenergic receptors also in other "responders", including humans.

In the Office Action the Examiner points out that "β_3 inhibition may have a beneficial effect in later stages of heart failure" according to the prior art (page 12 of Office Action). The MOXCON study, combined with the data presented in the instant application strongly teaches away from the prior art the Examiner refers to. This will be addressed in more detail in response to **(E)** below.

The Examiner states: "In contrast, other references suggest that β_3 stimulation may have beneficial effect in later stages of heart failure by increasing nitric oxide synthesis, additional vasodilating effect on vessel tone might also contribute to decrease in the peripheral vascular

resistance and the afterload of the failing heart. Also, a local nitric oxide release in the myocardium…could enhance diastolic relaxation and reduce oxygen consumption, thereby improving cardiac status (Moniotte, p. 490, right column)".

Moniotte *et al.* provide no data or references to the literature that supports their speculations. Indeed, as explained in the following paragraphs, the widely accepted evidence at the time (2003) teaches away from the speculations the Examiner quotes.

Re: vasodilating effect and decrease in afterload: This hypothesis had already been tested in human clinical trials at the time. Vasodilators had been found to be ineffective and probably harmful, as reviewed (Klein *et al*, 2003, page 32F). A skilled practitioner would have been aware of this and placed no credence on the unsubstantiated speculations put forward by Moniotte *et al.* Subsequent trials have confirmed the earlier studies: Vasodilators are harmful in heart failure. In addition, when the literature is critically examined, the applicants are not aware of good evidence to indicate that selective β_3 stimulation causes vasodilation in humans.

Re: local nitric oxide (NO) release in the myocardium: NO has complex effects on the myocardium. Moniotte *et al* extrapolated the effects believed to apply for normal myocardium to the failing myocardium. Cotton *et al.* (2002) reviewed NO effects in the normal and failing heart. Their account for the normal heart concurs with the account briefly given by Moniotte *et al* (2003). However, Cotton *et al* (2002) also review evidence indicating that effects in the failing heart are likely to be very different. They conclude (p. 565, last line lower right column bridging to p 566, top left column): "given that the presence of oxidative stress often dramatically alters actions of NO (generally from beneficial to deleterious). There is still a long way to go before a better understanding of the NO pathway in heart failure could be translated into clinical therapeutic advances".
The evidence at the time supports a critical difference in effects of NO in the normal and failing heart proposed by Cotton *et al.* (2002) while it teaches away from the speculations of Moniotte *et al.* (2003): A careful *in vivo* human study had shown that inhibition of nitric oxide synthase (and hence an expected decrease in myocardial NO) had improved function in the failing heart but had little effect in the normal heart (Hare *et al* 1998). The extrapolation

of effects of NO in the normal heart to the failing heart that Moniotte *et al.* proposed would have been regarded as too simplistic to have any credibility amongst practitioners skilled in the art.

The Examiner has formed the opinion that the working examples presented in the specification do not resolve the conflicting and contradictory data known in the prior art (page 12 of Office Action). It is respectfully submitted that data is not provided to support the speculations from the literature that the Examiner quotes and that some of the speculations teach away from the evidence that was available. Support for the treatment of heart failure with β_3 adrenoceptor agonists is unrelated to the literature the Examiner cites. The evidence supporting use of β_3 adrenoceptor agonists to treat heart failure on the basis of the present invention is presented below.

The Examiner points out discrepancies between *in vitro* studies on human ventricular and atrial tissue (starting last paragraph on page 11 of Office Action. As discussed by Gauthier's group, there are a number of technical deficiencies in the published studies on human tissues that preclude interpretation (Audigane *et al.* 2009, top left column, page 401). In addition, regardless of such deficiencies with the published studies, atrial function is not regarded of importance in the syndrome of human heart failure; it's the function of the left ventricle that is important. To illustrate this, "atrial fibrillation" with no atrial pumping function is very common and does not *per se* cause heart failure (it may be a complication of heart failure, though).

In **Point (E)** of the Office Action the Examiner was concerned about the quantity of experimentation needed to make or use the invention. The working examples in the instant specification describe how β_3 adrenoceptor agonists, including norepinephrine, stimulates the Na^+-K^+ pump, essentially the only export route for Na^+ in cardiac myocytes. It had been widely accepted that norepinephrine levels are elevated in heart failure. It was widely believed that the raised norepinephrine levels were causally related to <u>harm</u> in heart failure and that a reduction in levels would be beneficial (see for example articles by van Veldhuisen and Poole-Wilson (2001) and Coats (1999), well known opinion leaders on the treatment of heart failure). Based on this widely held view the MOXCON trial was conducted on the use

of Moxonidine, a drug that was known to markedly reduce norepinephrine levels in heart failure patients.

The MOXCON trial showed a large increase in mortality in Moxonidine treated patients compared with controls (Cohn *et al.*, 2003, Figure 1, page 663) with the main modes of death being "sudden" and "pump failure" (Table 3 same page) that are classical modes for heart failure. The reason for the unexpected adverse outcome was not established (Cohn *et al*, 2003; Coats, 1999). Cohn and his co-authors as well as Coats are among the World's foremost experts on heart failure. They had no reason to suspect that reducing norepinephriine-dependent β_3 adrenoceptor stimulation could have had an adverse pathophysiological effect in the MOXCON study nor did they or any others publish such a view subsequently. The present invention provides a good explanation for the adverse outcome as outlined below.

Given the widely accepted adverse pathophysiological role of raised cardiac myocyte Na^+ levels in human heart failure and animal models (as reviewed, Pieske and Houser, 2003; Pogwizd *et al.* 2003) and the effect of the proven heart failure treatments with angiotensin converting enzyme inhibitors and aldosterone antagonists (ESC guidelines, 2008; Klein *et al*, 2003) to increase activity of the Na^+-K^+ pump (Hool *et al.*, 1995; 1996; Mihailidou *et al.*, 2000) it reasonable to expect that pharmacological activation of the Na^+-K^+ pump would be useful. The adverse effect of reducing levels of the β_3 receptor agonist norepinephrine in the MOXCON study imply a beneficial effect of stimulation of Na^+ export mediated by the receptor that could not have been anticipated without the information provided by the examples in the instant application.

The norepinephrine levels reported in the MOXCON study were below levels expected to saturate and maximally activate the β_3 adrenergic receptor according to the human receptor's published affinity constants for norepinephrine. Based on the present invention, a selective synthetic agonist can therefore be administered therapeutically with a reasonable expectation of success. It is important to note that Moxonidine was used in human clinical trials on the basis of an association only between raised norepinephrine and heart failure. In contrast, use

of a selective agonist as described in this application would have the support of a mechanistic rationale that is now understood on the basis of the examples presented.
In summary, the Applicants respectfully submit that the instant specification does fully enable one skilled in the art to use the invention as claimed and that the requirements of 35 USC 112 paragraph 1 have been met..

The rejection under 35 USC 102 of claims 3 and 5 is moot in view of the amendment of claim 3, on which claim 5 depends, to incorporate the subject matter of claim 4 against which no rejection was made under this provision.

So far as the rejection of all claims under 35 USC 103 over Bush WO 02/94820 in view of Moniotte and the ESC Guidelines are concerned, it is respectfully submitted that the Bush reference cannot bear the weigh the examiner puts on it.

Bush *et al.* claim usefulness of SAM II in the treatment of heart failure and 23 other, seemingly random conditions. The compound was developed for the purpose of treating obesity or diabetes (see "Background of the Invention" page 1), and a compound was sought that would activate β_3- but not β_1 and β_2 receptors (top of page 2). Most of (WO02/94820) describes details and alternative routes of SAM II synthesis. Two functional examples only are provided.

In the first example it is shown that β_3 receptors expressed in Chinese hamster ovary (CHO) cells can mediate the production of cAMP in the CHO cells when they are exposed to 4 different salts of SAM II (cAMP production expressed as % of the response elicited by isoproterenol in Table 6, page 24). If these results were to be extrapolated to efficacy of SAM II in the treatment of heart failure, the example provided would, in the opinion of the Applicants, clearly teach away from such use; it is widely appreciated that activation cAMP-mediated by β_1 agonists (increase cAMP production in myocytes (see Fig 4 on page 684 of Gauthier *et al.*, *Can J Physiol* 2000) and phosphodiesterase III inhibitors (inhibit cAMP breakdown) is harmful in heart failure (see Klein *et al.*; 2003 page 31-32)

The second example included in Bush describes how SAM II has no effect on the beating rate

of 3 isolated rat atria. While these experiments support a lack of unwanted β_1 and β_2 receptor activation, the demonstrated absence of a cardiac effect has no implication for usefulness in the treatment of heart failure (claim #21 on page 25).

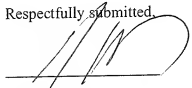
To summarize, one example provided in Bush teaches away from use of SAM II in heart failure. The other has no implications at all. A practitioner cannot use Bush *et al.* to support use of a β_3 receptor agonist in the treatment of heart failure with any expectation of success. If there is any implication at all, it teaches away from the claims made in the present application.

In view of these comments regarding Bush *et al.* and the earlier comments herein regarding Moniotte *et al.* and the ESC Guidelines, the Applicants submit that the claimed invention is not obvious in light of any of the cited documents, either considered individually or in any combination.

Accordingly, it is submitted that the claims under consideration all meet the requirements of 35 USC 103 and it is respectfully requested that these rejections be withdrawn.

It is therefore submitted that this application is in order for allowance and an early action to this end is respectfully solicited.

Respectfully submitted,



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